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Some Observations on the Early History of DNA

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It is both a pleasure and a privilege to participate in this official opening of the Institute of Human Virology. I have assumed that my role in this "Celebration of Biomedical Science" is to touch upon some of the historical aspects of the origins of the current revolution in biology that contributes so heavily to the progress of research in all branches of biology today. Specifically, of course, my comments will relate to the research that provided the first experimental evidence of the genetic role of DNA.

Since the existence of what we now call DNA in the nucleus of mammalian cells was first discovered by Friedrich Miescher in 1869, when he isolated a material that he termed "nuclein", it is reasonable to ask why it took until almost the middle of the next century for its biological function to be recognized. Obviously, many factors were involved in this protracted period of slow progress. However, one should recall that all of the relevant biological sciences - biochemistry, cell biology, and genetics - were in relatively early stages of modern development. The initial clarification of the nature of nuclein came only gradually, leading first to a better understanding of the two separable components - protein and nucleic acid - and then to the identification of the purines, pyrimidines, and sugars that provided the basic units of nucleic acid, along with the phosphate, the high content of which Miescher had noted from the start. This process continued well into this century, with much of the later work taking place in Albrecht Kossel's laboratory in Germany and P.A.

Levene's in the U.S. Kossel received the Nobel Prize in 1910, in part for this work, and the fact that some confusion about the nature of nucleic acid still existed at that time is reflected in the brief citation used in the listing of the Nobel Prizes in Physiology and Medicine, which reads: "Albrecht Kossel in recognition of his contribution to our knowledge of cell chemistry made through his work on proteins, including the nuclear substances." It was not until 1929, sixty years after Miescher's discovery, that Levene reported the correct identity of the final component of DNA, the sugar that gives it its name - deoxyribose.

This glacial pace of the early research does not mean that no one was moved to suggest that the nucleic acid might have a genetic role. It would have been surprising if this thought had not occurred to some of the workers struggling with this newly recognized component of the cell nucleus. One of those who proposed this possibility in print was an American, Albert Mathews, who worked with Kossel in Marburg. Another was the American cytologist, E.B. Wilson, who wrote in his book, *The Cell in Development and Heredity* in 1895 that "Chromatin is known to be similar to, if not identical with, a substance known as nuclein, which analysis shows to be a tolerably definite chemical compound composed of nucleic acid and albumin. And thus we reach the remarkable conclusion that inheritance may, perhaps, be effected by the physical transmission of a particular compound from parent to offspring." He later focused on the nucleic acid as the likely component. Both Mathews and Wilson retracted their views in later

publications. Mathews was influenced by his continued chemical studies of nuclei from various sources that led him to conclude that the nucleic acids were pretty much the same from all sources while the associated proteins were highly variable. Wilson was swayed not only by Mathew's data but also by histochemical evidence of others that was interpreted as showing that the nucleic acid disappeared from the nucleus during certain phases of the cell cycle. Thus, flawed experimental observations were responsible for ending speculation about the possible genetic role of DNA.

A later example of a remarkable view on this subject was brought to my attention a few years ago by the late Bernard D. Davis. In a reprinting of Emil Fischer's *Aus Meinem Leben* that appeared in 1987, Bernard Witkop in his prologue quotes a passage from a paper published by Fischer in the *Berichte* in 1914. This quote, which is in discussion of work on the synthesis of methylated purines, runs as follows in Witkop's English translation:

"With the synthetic approaches to this group we are now capable of obtaining numerous compounds that resemble, more or less, natural nucleic acids. How will they affect various living organisms? Will they be rejected or metabolized, or will they participate in the construction of the cell nucleus? Only the experiment will give us the answer. I am bold enough to hope that, given the right conditions, the latter may happen and that artificial nucleic acids may be assimilated without degradation of the molecule. Such

incorporation should lead to profound changes of the organism, resembling perhaps permanent changes or mutations as they have been observed before in nature."

Thus, in this extraordinary bit of premature speculation, Fischer was not only thinking in terms that sound like gene transfer but hoping that it could be accomplished with synthetic material. This could be the earliest expression of the idea of genetic engineering. I know of nothing to indicate that he returned to this theme again during the few remaining years of his life.

Gradually, any expression of the view that nucleic acids might have a genetic function practically disappeared during the 1920s and 1930s. The idea that nucleic acids were too simple and repetitive in structure to have specificity had become the general view, aided by Levene's tetranucleotide theory which, without there being much evidence to support it, had been interpreted as meaning that this was the fundamental unit of the molecule with the four nucleotides arranged in the same order. When the nature of genes was even mentioned during the 1930s, it was usually with a statement such as: if genes are composed of a known substance, there are only the proteins to be considered. Dissenters from this view were indeed rare. One of these was Jack Schultz of the Fox Chase Cancer Center who, writing on the nucleoprotein nature of the gene in 1941, stated that there was too little information to conclude that nucleic acids were monotonously uniform and that much more work had to be done before one could exclude the

possibility of specificity of this chromosomal component.

At the time that we reported the conclusion of the long search for the identification of the chemical nature of the pneumococcal transforming substance in 1944, suggesting that the evidence indicated that it was DNA, the predominant reception was disbelieving or at least very skeptical, primarily because of this generally held view of the simplicity of nucleic acids. The evidence that we had accumulated in favor of DNA was certainly diverse and strongly supported the conclusion, even though we had not yet been able to demonstrate the rapid inactivation of transforming DNA with a purified DNase, since DNA enzymology had lagged behind that for proteins and RNA. It required over a year more work before we could prepare a suitable DNase for this final piece of evidence. Even this did not satisfy all of the skeptics, and except for a few workers who accepted the evidence at the outset and based their own studies on the conviction that it was correct, the conversion was a gradual process that took a few years.

In addition to the prevalent idea that the activity of our DNA might be due to a trace of contaminating protein, there were those who proposed that we were not dealing with gene transfer, and that our material was merely activating a latent gene in the responding cell. This was akin to the idea that was entertained in the Avery lab during the early years of research on transformation: that the pneumococcal cell had the capacity to synthesize many of the specific capsular polysaccharides and what the trans-

forming substance was doing was to select and activate the production of the correct one. There were a number of problems with the idea, but the most important was that one had to account for the fact that the "activator" appeared to be replicated with each division of the cell, since the transformed pneumococci were as good a source of the active substance as the original strain of pneumococcus. This evidence for self-replication did a lot to reinforce the view that genetic transfer was involved. Of course, in the late 1940s the work of Hotchkiss and others established that a number of other attributes of pneumococci, such as antibiotic resistance and expression of specific proteins, could also be transferred by DNA, thus making this hypothetical reservation irrelevant.

As has been frequently noted, our 1944 paper carefully avoided any direct statement that the DNA was acting by the process of gene transfer, and the fact that the active principle was replicated in the transformed cell was reported but not emphasized with respect to its implications. The discussion was written by Avery, with a few additional inputs from MacLeod and me, but there is no doubt that we were inclined to be less reserved in our interpretations than Avery. However, we were happy to have him agree to at least publish the evidence, in view of his reluctance to move ahead without resolving all possible doubts.

The discussion of the paper included a few paragraphs on the various interpretations that had been advanced concerning the

nature of the transformation phenomenon. It was noted that it had been interpreted from the genetic point of view, citing Dobzhansky's view in his 1941 book, *Genetics and the origin of species*, and a quote that I had found in R.A. Gortner's *Outlines of Biochemistry*. With some relevance to this official opening of the Institute of Medical Virology, the discussion then pointed out that "Another interpretation of the phenomenon has been suggested by (Wendell) Stanley who has drawn the analogy between the activity of the transforming agent and that of a virus." He had raised this point in his chapter of Doerr and Hallauer's *Handbuch der Virusforschung* , published in 1938, where he wrote that "This phenomenon is virus-like, and it is because of this and the fact that it may become important from the standpoint of the chemistry of viruses that a discussion is included here".

Stanley had continued to hold this view, and in 1946 he invited Avery to participate in a symposium on Biochemical and Biophysical Studies on Viruses held in connection with a meeting of the American Chemical Society in Atlantic City in April. As was usual in those days, Avery declined the invitation and offered me as his substitute. Stanley's letter to Avery acknowledging the substitution is still in my files, and it states "I am delighted to have your letter of January 5 and to know that Dr. McCarty will present a paper at the meeting . . . I am sorry that you do not wish to have your name to appear on the program with Dr. McCarty, but I am sure that I understand your view point." I took this as an indication that I could do my own thing.

The symposium was not published but I had prepared a full typescript and still have a copy available. I had been interested in the virus story, especially as it was becoming clear that they all were likely to be nucleoproteins. The recent work of Cohen and Stanley showing that the crystalline tobacco mosaic virus had a high molecular weight RNA component had intrigued me, and I'm sure that I interpreted its presence somewhat differently from Stanley. Once I had prepared a highly active DNase I had taken advantage of my access to the Horsfall laboratory at Rockefeller to test it on the activity of a couple of viruses, and found that the enzyme had no effect on the infectivity of the influenza virus or the pneumonia virus of mice, even when the enzyme was used in amounts that would totally depolymerize native DNA in seconds. Of course, I had no idea at that point what kind of nucleic acid these viruses contained, but my approach to the symposium paper was influenced by this experience nevertheless.

My paper was entitled "Chemical Nature and Biological Specificity of the Substance Inducing Transformation of Pneumococcal Types" and for the most part summarized the DNA story, on the assumption that it would not be familiar to much of the audience. In recognition of Stanley's view that the phenomenon is virus-like, however, I noted in passing that "The ease with which the pneumococcal transforming substance can be inactivated by enzymatic action stands in direct contrast to the accumulated experience with animal and plant viruses and bacteriophage, which have been shown to be highly resistant to inactivation by

nucleases, and in most instances by other enzymes as well. It may be that this is a fundamental difference between the transforming substance and the viruses, a difference that has already been suggested by the apparent absence of protein and of serological activity." I returned to this topic in my final paragraph in order to make an additional point. This paragraph reads as follows:

" It will be observed from the foregoing discussion that while the pneumococcal transforming substance is virus-like in certain of its properties, there is some evidence inconsistent with its classification with the viruses, despite the diversity of this group of agents. However, if one accepts the validity of the view that the biological specificity of the transforming substance is the property of a desoxyribonucleic acid, the results of the present study serve to focus attention on the nucleic acid component of the virus nucleoproteins. In addition to its probable role in the self-reproduction of the virus molecule, the nucleic acid moiety may carry a specificity which is a determining factor in the ultimate structure of the virus." If Dr. Stanley heard these remarks, he apparently did not take them seriously.

There was a sequel to this story almost twenty years later in connection with the ceremony for the dedication of the Avery Memorial Gate at the Rockefeller Institute in September 1965. I served as one of the organizers of this affair, and in reply to my letter inviting him to participate in the ceremony, Dr. Stan-

ley reminded me of the comment that he had made in the Doerr and Hallauer *Handbuch* about pneumococcal transformation being virus-like. He went on to say that "Tom Rivers gave me a pretty bad time, for I had asked him to look over my chapter and he felt the section on 'The transforming agent of the pneumococcus' should be deleted, for, as he put it, it had absolutely nothing to do with viruses. Much argument could not convince him, but I felt so strong about it that the section was left in." It occurred to me that I had taken Rivers' side in the argument at the Stanley 1946 symposium with some additional evidence to back it up.

The several talks given at the Avery Gate ceremony were never published, but Stanley wrote a paper that appeared in 1970 in the *Archives of Environmental Health* entitled The "Undiscovered" Discovery. A final footnote in this article stated: "This communication was developed from notes which were used in connection with a speech I presented on the occasion of the dedication of the Avery Memorial Gateway . . ." This paper included his reminiscences about his contacts with the Avery lab while the work was going on there, a summary of the early studies on nucleic acids, beginning with Miescher, and a detailed record of our work and the 1944 paper.

His title, of course, referred to the frequently discussed lag in the general acceptance of our findings, and his term, "undiscovered", has a similar implication to Stent's "premature". He does note the research of Chargaff establishing the diversity of DNAs from different sources that followed promptly in the late

1940s, but does not seem aware that Chargaff is one of the most notable of those who did accept our conclusions, since he acknowledged on more than one occasion that he had changed his major research effort to nucleic acids on the basis of our paper.

The final section of the Stanley paper is entitled **An Apology**. He begins by noting that he knew by 1936 that his crystalline tobacco virus was a ribonucleoprotein, and that it was this and his knowledge of Avery's work on transformation that led him to include his remark about the active agent being virus-like, over River's objections, in the *Handbuch der Virusforschung*. He then adds the following: "I was also interested in the RNA of tobacco mosaic virus. In 1942, Cohen and I reported the isolation of this RNA with an unusually large molecular weight and we reached the conclusion 'that the nucleic acid exists in thread-like molecules, the length of which is that of the intact virus molecule.' It is obvious that despite my 1938 writings, I was not impressed with the 1944 discovery by Avery, MacLeod, and McCarty or I would have prepared high molecular weight tobacco mosaic virus-RNA once again and tested it for virus activity despite the fact that RNA was not suspected of having genetic activities. It remained for Fraenkel-Conrat to do this important experiment in my laboratory 14 years later." Stanley then noted factors, such as his war activities that may have explained his failure to recognize the full significance of transforming DNA, but ends with the comment: "But there should have been time for me to accord proper early recognition to the discovery of transforming

DNA in 1944, and for my failure to do this I apologize."

I will close with a note on the reaction of another virologist who had a role in the publication of our 1944 paper. This was Peyton Rous who as Editor of the *Journal of Experimental Medicine* had been asked by Avery to give our paper full review, just as if it had come from a stranger. Rous complied and brought the manuscript back to us for discussion covered with his characteristic pencilled notes that raised a variety of questions, all of which we responded to. Unfortunately, this marked copy was lost. I remember a number of his comments, however, one of which dealt with the quote that I had introduced from Gortner's textbook of biochemistry. Gortner had referred extensively to an article by J.B. Leathes, entitled "Function and Design" that had appeared in *Science* in 1926. The part that had caught my eye was in discussion of the finding that nucleic acids formed approximately 40 per cent of the solid components of the chromosomes, and Gortner had quoted Leathes as saying that if we consider that into these chromosomes "are packed from the beginning all that preordains, if not our fate and fortunes, at least our bodily characteristics down to the color of our eyelashes, it becomes a question of whether the virtues of nucleic acids may not rival those of amino acid chains in their vital importance". Rous pointed out that this was pure speculation and added nothing to our thesis. It was removed, but the reference to it was retained.

Rous's view of the work in general was not known to me until years later after his death. His copy of the reprint of the

published paper, which after his death had come into the hands of a colleague at Rockefeller, had a pencilled note to his secretary written in his hand on the cover, reading: "File under genetics."